Textbox 4.1

A shift in the use of aminoglycosides following the ceased use of zinc oxide

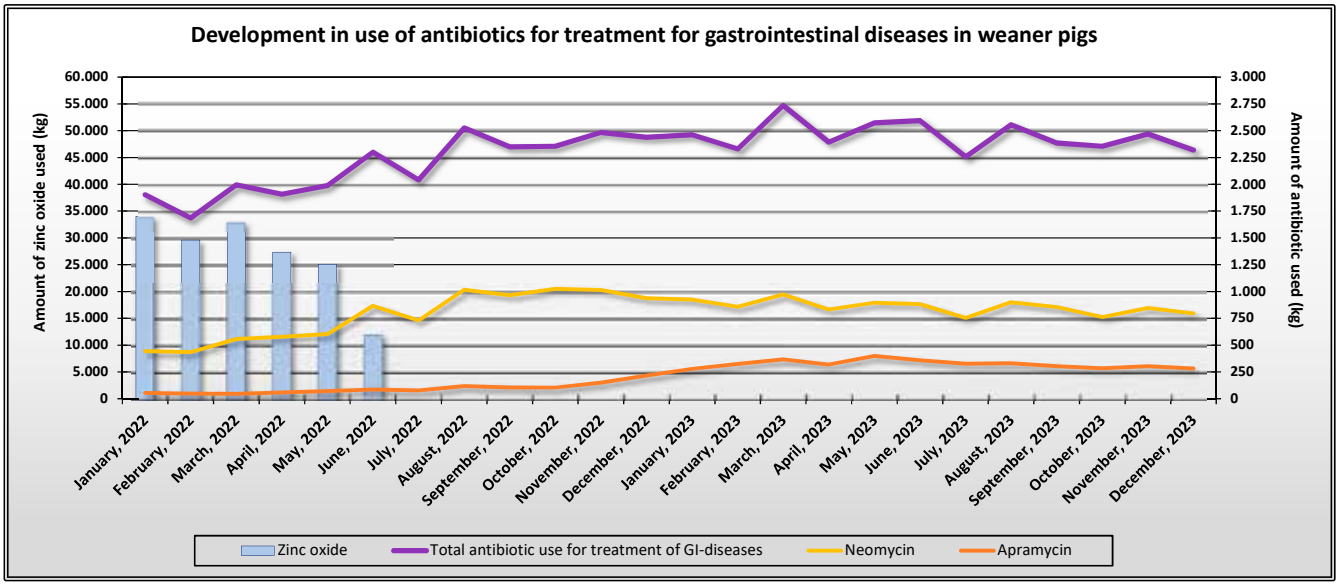
The use of zinc oxide in Veterinary Medicinal Products (VMPs) was banned across Europe from June 26th 2022, due to a consideration of the risks for the environment. Prior to this, VMPs containing zinc oxide had been used to control the occurrence of post-weaning diarrhea in piglets. The political ambition in Denmark was that the ban of zinc oxide should happen without an increase in antimicrobial use. While the Danish pig industry investigated strategies to find suitable alternatives for managing post-weaning diarrhea in the years leading up to the ban, the complete discontinuing use of zinc oxide led to an acute rise in the use of neomycin in 2022; as described in the DANMAP 2022 report.

Neomycin is a first-choice antibiotic for treating post-weaning *E. coli* associated diarrhea in the Danish national antibiotic treatment guidelines for pigs and was used in some capacity for treating post-weaning diarrhea before the ban on zinc oxide. With the increased use came increasing problems with resistance towards neomycin, which led to an increased use of apramycin towards the end of 2022 and the beginning of 2023. In 2023, the levels of use of both neomycin and apramycin have stabilized, but to a higher level than prior to the ban of zinc oxide.

The increase in neomycin resistance and increased use of apramycin can be problematic, due to the impaired effectiveness and unfavorable alternative antibiotic treatment options. A range of new initiatives to decrease antibiotic use in pig production have been launched in 2024, as specified in the political food and veterinary agreement 2024-2027 and in the Danish Veterinary and Food Administration's 'National action plan on Antimicrobial Resistance in animals and food' for 2024-2027.

The Initiatives includes updates of the national antibiotic treatment guidelines, new thresholds for the Yellow Card initiative, focusing on weaned piglets up to 30 kg and an investigation of a benchmarking-model for veterinarians along with a continued focus on investigating alternatives to antibiotic treatment.

Figure 1 Development in use of antibiotics for treatment for gastrointestinal diseases in weaner pigs DANMAP 2023



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Textbox 4.2

Veterinary medicines and antibiotic resistance

In recent decades, veterinary medicine has focused on a One Health approach that integrates considerations regarding the health of both animals and humans. This has led to new guidelines for the responsible use of antibiotics in animals, aiming to reduce the development of resistance. As part of these efforts, the EU's Veterinary Medicines Regulation (2019/6) was implemented in 2022 with the goal of harmonizing the use of veterinary medicines, including reserving critical drugs for humans and generally ensuring responsible use. Veterinarians support these initiatives, but unfortunately, certain provisions in the regulation, especially Article 106, cause significant challenges for many veterinarians, which may impact a responsible use in a negative way. Veterinarians are required to prescribe medications strictly according to the Summary of Product Characteristics (SPC) which, depending on the available drugs, limits their ability to tailor treatments to individual animals or herds.

Outdated product summaries
One of the biggest challenges is that the SPCs for many veterinary medicines, such as older narrow-spectrum antibiotics, are not updated in line with new knowledge. This forces veterinarians to follow outdated dosage recommendations and treatment durations, which can lead to unnecessary use of medication and, consequently, increase the risk of antibiotic resistance. Marketing authorization holders rarely see a benefit in updating SPCs to reflect new indications, agents, or treatment regimens, as this is resource-demanding, and the risk is that more veterinary medicines will be withdrawn from the market.

Failing access to important medicines
The regulation aims to reduce administrative burdens, strengthen the market, and promote innovation in the veterinary pharmaceutical industry, but there are still challenges in ensuring a stable supply of necessary veterinary medicinal products. And although EU harmonization is intended to reduce discrepancies, in several countries, including small markets like Denmark, shortages of essential products such as certain vaccines and narrow-spectrum antibiotics have been reported. This can drive veterinarians to use broad-spectrum antibiotics instead, increasing the risk of resistance. In addition, Danish veterinarians still face difficulties accessing products like autogenous vaccines, which their colleagues in other EU countries can use. This, combined with the lack of marketed vaccines risks compromising preventive efforts and leading to more disease outbreaks that require antibiotic treatment.

Consequences for animal treatment
Article 106 of the Veterinary Medicines Regulation thus creates practical problems in many treatment situations, including in pig herds. For instance, the strict interpretation of SPCs for medications may result in longer antibiotic treatments with higher doses, even though new empirical knowledge and evidence shows that lower doses for shorter periods are just as effective. This challenges the goals of reducing antibiotic use in this sector.

Similar challenges are seen with companion animals and horses, where veterinarians traditionally base treatment durations on clinical signs, on translating experience from human medicine and scientific guidelines to minimize antibiotic use and unnecessarily long treatments. This is no longer possible under the current requirements, affecting both animal health and welfare.

The need for flexibility
There is a great need to hand back to veterinarians the flexibility to deviate from SPCs when new research supports it. This will ensure better treatment for animals and reduce the risk of antibiotic overuse, which is a major threat in the fight against antimicrobial resistance. Veterinarians want to base their decisions on the latest scientific knowledge rather than being bound by outdated guidelines and rigid legal requirements. This flexibility will help reduce antibiotic use and lower the risk of resistance development, which affects both animals and humans.

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Textbox 5.1

International approach to improve supply of antibiotics

What makes a market vulnerable? Which factors need to be considered when trying to strengthen a market? It seems like repeated shortages and length of shortages are not necessarily a sign of nor does sales volume necessarily correlate with an elevated risk of withdrawal[1]. The deregistration problems and shortages issues must then have roots elsewhere. Some of them will be mentioned here, but it's a multifactual problem.

Antimicrobial stewardship programs aim at promoting rational prescribing of antimicrobials in accordance with treatment guidelines. Thus, the programs will result in decreased use of antibiotics, resulting in a smaller and less attractive market. However, antimicrobial stewardship programs are essential[2] in the fight against antimicrobial resistance and initiatives to solve supply chain problems therefor need to take this into account.

Development of new antibiotics is not profitable, as they often are to be preserved for last treatment option. On the other hand, old antibiotics, e.g. phenoxymethylpenicillin have difficulties getting registered in new markets because updated legislation has new requirements on indication and doses.

All countries have individual legislation on registration of medicinal products. Different legislative requirements make the production difficult, costly and inefficient. A report published by PLATINEA showed that within the Nordic market (Finland, Norway, Sweden and Denmark) phenoxymethylpenicillin is marketed in 10 different strengths, 41 different package sizes – of which 33 packages are only marketed in one single country[3]. The Nordic market combined makes up a quarter of the Italian antibiotic market – thus a high fragmentation as seen with phenoxymethylpenicillin in a small market must be accounted as a major risk factor for future shortages.

EU-JAMRAI-2 WP9 ACCESS

JAMRAI-2 is a One Health initiative and the biggest AMR investment from the EC EU4Health: 50 million Euros, 30 countries (27 European countries and Iceland, Norway and Ukraine), more than 100 partners. From 1st of January 2024 till 31st of December 2027 the countries will work together to attack the AMR-problems from different perspectives. Ten work packages are covering the update or development of National Action Plans (NAP), including important activities such as Infection Prevention and Control (IPC) programmes with the implementation of pilot studies, surveillance of resistance in humans, animals and the environment and access to antibiotics and vaccines.

EU-JAMRAI 2 receives funding from the European Union's EU4Health programme under grant agreement n°101127787.

Work package 9 on ANTIBIOTIC ACCESS (WP9) has 14 participating countries with the objective to help the member states in regards to strengthened/improved access to selected AMR-related products (antibiotics and vaccines) both for human and for veterinary use. WP9 will primarily focus on country-specific needs and demands.

The work package consists of several tasks and subtasks. The first task for each participating country is to develop a national list of vulnerable AMR-related products (antibiotics and/or vaccines). The aim is to focus on older, narrow-spectrum antibiotics used as first-in-line treatment or to treat a small group of critically ill patients and vaccines used to prevent bacterial infections; the products chosen must have a vulnerable supply and include both human and veterinary products.

Once the priority products have been chosen, each country must try to look at the demand and supply barriers. What are the main reasons for clinicians not to prescribe drug A? Is the drug not registered? Is it a more expensive choice? Does the country experience lots of shortages? Why is drug B not available in the country? Could harmonization between different participating countries be an option to increase the demand of a drug needed for the participating countries?

[1] Läkemedelsverket/ SWEDICH MEDICAL PRODUCTS AGENCY: "*Indicators that reveal antibiotics at risk of withdrawal*"
[2] amr_2017_action-plan_0.pdf (europa.eu)
[3] 240212_Mappingtheantibioticmarket_PLATINEA.pdf (uu.se)

continued ... Textbox 5.1

When mapping the demand and supply barriers each participating country will work in close collaboration with WP9 but also national stakeholders to look and implement possible interventions to strengthen the supply of the prioritized products. The lists from all participating countries will be compared, and countries with similar demands will be encouraged to work together to over time achieve a strengthened access to and more secured supply of their chosen products.

An important aspect of the work package is thus to make individual countries aware of similar challenges and bring common demands as well as commonly detected causes of impacted supply to the attention of the EU for a better understanding and possible cross-country interventions.

In Denmark, hopes are high for better future supply of many of the small-spectrum penicillins, particularly in pediatric formulations, an interest that has been brought up also by other countries. The synergy that stems from developing demand lists and supply challenges in parallel should not be overlooked. Bringing together technical as well as political levels of different countries on these topics brings the advantage of attracting more attention to the problem as well as solving it. We thus find that EU-JAMRAI II offers an opportunity to make a change, not only within a country but across.

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EU-JAMRAI Joint Action Antimicrobial Resistance and Healthcare-Associated Infections

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Textbox 5.2

HALT 4 – An audit on infections and consumption of antibiotics among residents in Danish nursing homes

HALT 4 (https://hygiejne.ssi.dk/overvaagning/halt-4-projekt) (Healthcare Associated Infections in European Long Time Care Facilities 4) is a European project managed by ECDC (The European Center for Disease Prevention and Control).

In 2023, the National Center for Infection Control (CEI) at Statens Serum Institut coordinated an audit (HALT 4) on the prevalence of infections and consumption of systemic antibiotics (incl. antifungal and antiviral agents) among residents in Danish nursing homes. This was performed in collaboration with local personnel responsible for infection prevention and control (IPC) and staff at participating nursing homes in the periods May to June and August to November.

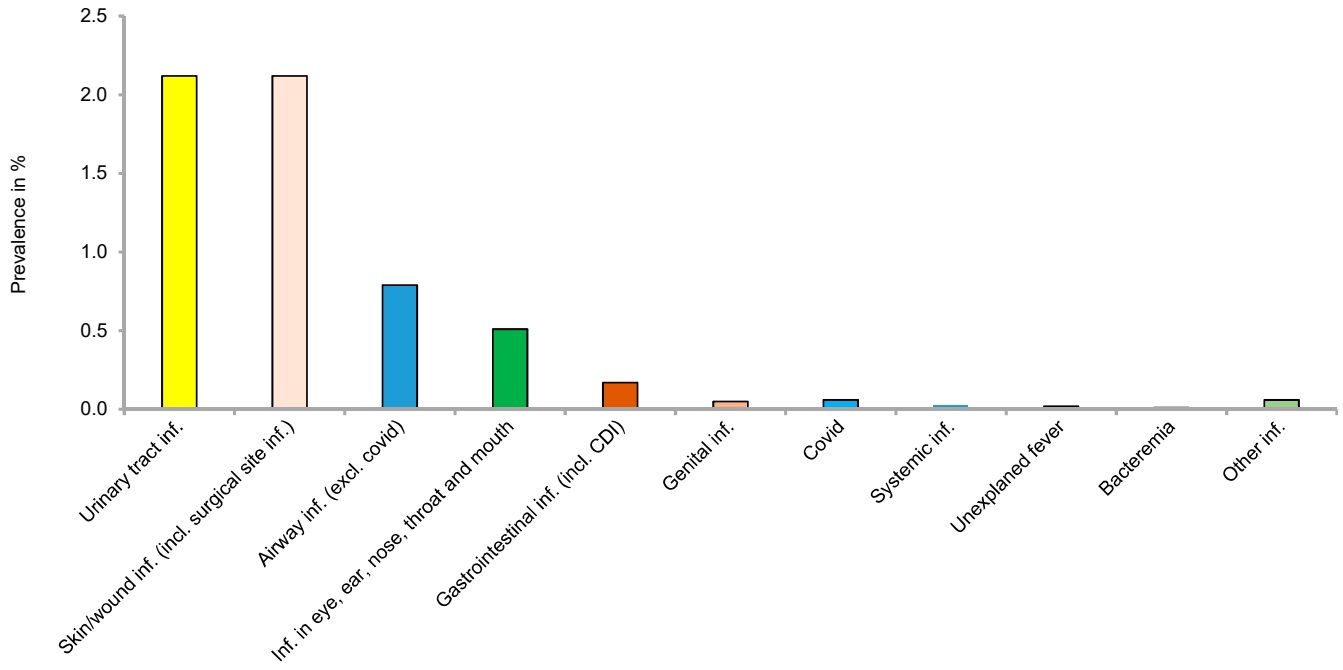
Purpose

- To focus on IPC, infections and consumption of systemic antibiotics and/or antifungal and/or antiviral agents in nursing homes
- To increase knowledge about the above conditions and the resources used on IPC in nursing homes
- To elucidate the conditions in nursing homes, both nationally and internationally, in order to be able to target future preventive interventions.

Method

CEI invited by mail and newsletter announcement all identified Danish nursing homes and all Danish municipalities and regions to participate. Participation was voluntarily and not randomized. Data collection and registration were performed by regional and/or municipal IPC personnel and/or by local staff at the participating nursing homes. Data were collected and recorded on one given day for each participating nursing home. Therefore, results represent a single point in time and do not provide information on a larger temporal scale.

Figure 1 Distribution of the various infections DANMAP 2023



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Results

In all, 294 nursing homes from 47 municipalities from all five regions participated, amounting to approx. 30% of the approx. 950 nursing homes in Denmark. Out of a total of 11,909 residents, 11,751 were included. 36.4% of the residents were men and 63.6% were women. The average age was 83.3 years, and 49.9% of residents were 85 years or older ; 29.8% of the residents had been living in the nursing home for less than a year.

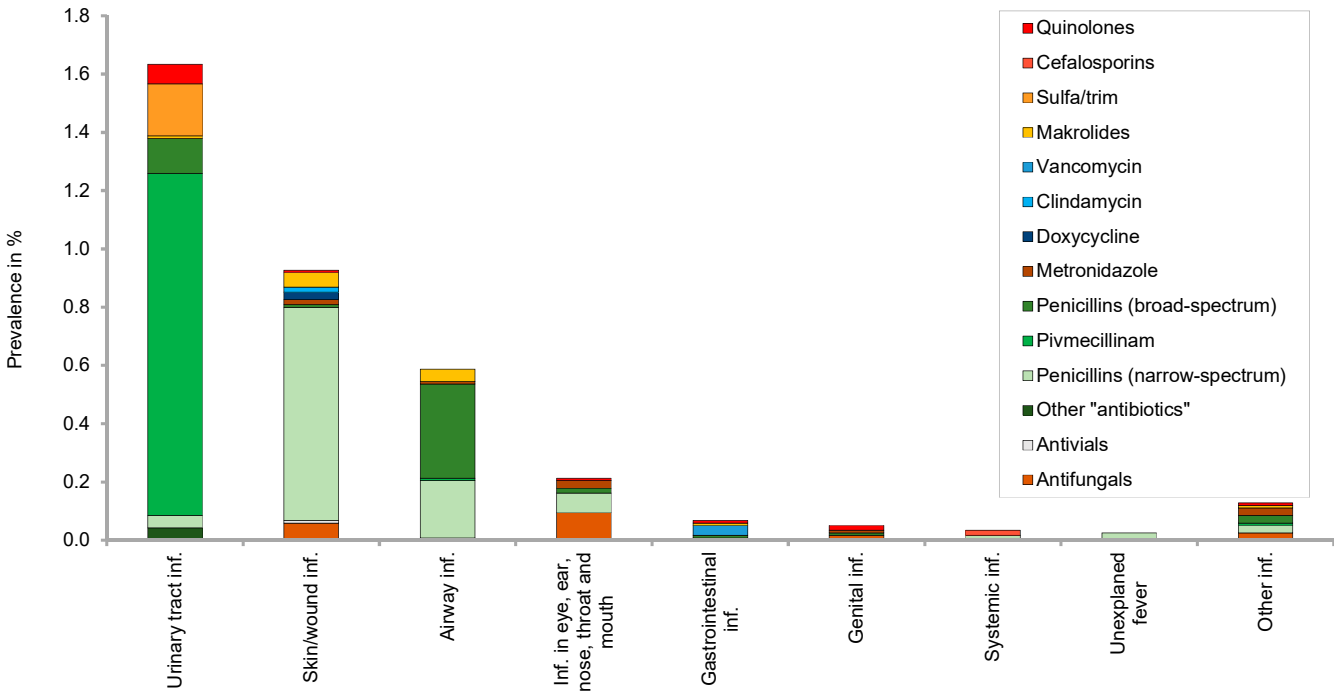
Infections

In total, 672 residents (5.72%) had an infection (defined as signs/symptoms of and/or received systemic antimicrobial therapeutic treatment against infection): 649 residents had one infection, 22 residents had 2 and one resident had three different infections, which amounted to a total of 696 infections. The distribution of infections can be seen in Figure 1.

Antimicrobial treatment

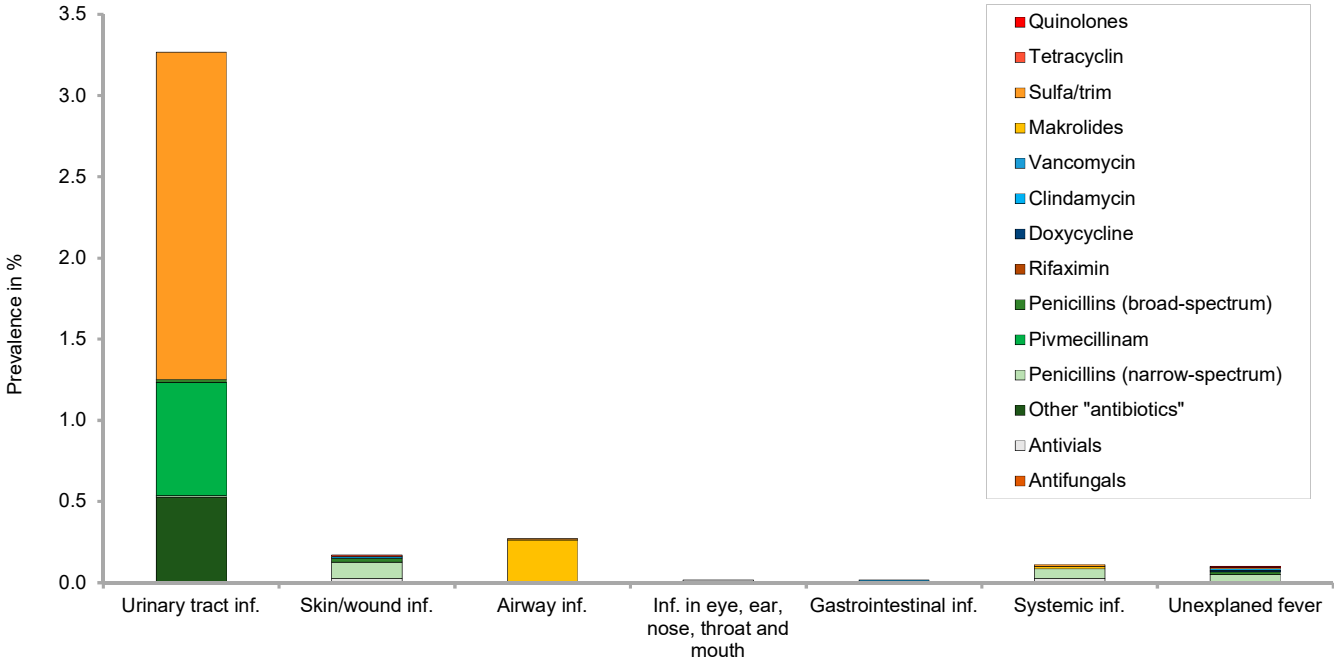
In total, 842 residents (7.17%) received systemic antibiotics and/or antifungal and/or antiviral agents. For 386 residents (3.28%), the indication was treatment of infection, for 445 (3.79%) it was prophylaxis, and for 11 (0.09%) it was both. Details are shown in Figures 2 and 3.

Figure 2 Distribution of 431 antimicrobials for systemic treatment of 403 infections DANMAP 2023



Quinolones include ciprofloxacin. Sulfa/trim includes sulfamethizole, sulfamethoxazole/trimethoprim, and trimethoprim. Macrolides include azithromycin. Broad-spectrum penicillins include amoxicillin and amoxicillin/clavulanic acid. Narrow-spectrum penicillins include dicloxacillin and phenoxymethylpenicillin. Other "antibiotics" include nitrofurantoin and methenamine. Antifungals include fluconazole. Antivirals include aciclovir, dolutegravir/rilpivirine and valaciclovir

Figure 3 Distribution of 465 antimicrobials for systemic prophylaxis in 457 cases DANMAP 2023



Quinolones include ciprofloxacin and moxifloxacin. Cephalosporins include ceftriaxone and cefuroxime. Sulfa/trim includes sulfamethizole and trimethoprim. Macrolides include azithromycin, clarithromycin, and roxithromycin. Broad-spectrum penicillins include amoxicillin, amoxicillin/clavulanic acid, and piperacillin/tazobactam. Narrow-spectrum penicillins include dicloxacillin and phenoxymethylpenicillin. Other "antibiotics" include nitrofurantoin. Antiviral agents include aciclovir. Antifungals include fluconazole, nystatin, and terbinafine

Comparing data from HALT 4 with data from the previously conducted and similar audit HALT 3 from 2017, the prevalences of residents with infection and both residents in therapeutic and prophylactic treatment are lower in HALT 4 than in HALT 3 (https://hygiejne.ssi.dk/overvaagning/halt-3---projekt). In particular, the prevalence of prophylactic treatment of urinary tract infection is significantly lower, see Table 1.

However, as the participating nursing homes in the two audits were not randomised, a comparison and a conclusion should be done with causion.

Table 1 Yearly number of isolates and patients DANMAP 2023

	HALT 4 – 294 perticipating nursing homes with 11,751 incl. residents	HALT 3 – 95 perticipating nursing homes with 3,346 incl. residents
Residents with infection	672 (5.72%)	227 (6.78%)
Residents in therapeutic treatment	392 (3.34%)	129 (3.86%)
Residents in therapeutic and prophylactic treatment	22 (0.19%)	5 (0.15%)
Residents in prophylactic treatment	446 (3.80%)	225 (6.72%)
Residents in prophylactic treatment of urinary tract infection	384 (3.27%)	213 (6.37%)

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Textbox 5.3

Infection Prevention and Control and prevention of Antimicrobial Resistance goes hand in hand

In Denmark there are numerous activities concerning infection prevention and control (IPC) and antimicrobial resistance (AMR) - both on the national and on the international level.

Across Europe as well as globally it is increasingly stressed that controlling AMR in human health must be based on aligning efforts within surveillance, antimicrobial stewardship (AMS) and IPC.

In June 2023, the European Union recommended to step up EU actions to combat antimicrobial resistance with a One Health approach. In the Council recommendations[1] they encourage member states to (extract):

- Have in place by the 14th of June 2024 and regularly update and implement National Actions Plans against AMR.
- Ensure that IPC measures in human health are put in place and continuously monitored to limit the spread of antimicrobial resistant pathogens.
- Ensure that measures are put in place in human health to support the prudent use of antimicrobial agents in health care settings including primary care and long-term care facilities.

To support the countries in stepping up the AMR actions the European Commision has invested 50 million Euros in EU-JAMRAI 2[2] which is a project under the EU4health programme[3].

EU-JAMRAI 2 (European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections) 2024 to 2027 seeks to implement concrete actions to monitor, prevent and effectively tackle AMR across human, animal and environmental health domains through a "One-Health" approach and to make Europe a best practice region. The project focuses on multiple areas, such as AMS, surveillance, awareness raising, capacity building, IPC and behavioral science. An essential part of the project is to support the countries in developing or updating their national AMR action plans with a focus on both AMR and IPC[4].

Among the 10 work packages (WP) in EU-JAMRAI 2, Denmark participates in WP5 (National Action Plans), WP6 (Antimicrobial Stewardship), WP7 (Infection Prevention and Control), WP8 (One Health Surveillance) and WP9 (Acces to antibiotics), see more in textbox 5.1. "International approach to improve supply of antibiotics" in DANMAP 2023.

WP7 is about improving IPC actions with a One Health approach. WP7 has tasks within the human, veterinary and environmental sectors and has a general focus on behavior change strategies to support further uptake of IPC recommendations. Several subtasks are described within the human activities: Development of frameworks for implementation of IPC competencies and prioritizing EU standards in IPC programs, support the participating member states and associated countries in the implementation of IPC core components, give access to an IPC toolbox and, finally, use peer-to-peer exchange programs including mentorships and observerships. Topics which constitute a challenge for IPC of today as e.g. lack of educated workforce, specialized care moving out of the hospitals and replaced by care at home, and IPC in the green transition will be included in the work. As part of the EU-JAMRAI 2 project the Danish National Center for Infection Control (CEI) in close collaboration with our national IPC partners aim at improving the acces to IPC knowledge and tools, strengthening IPC networking and knowledge-sharing, supporting pilot projects and other implementation activities. A general focus will be on how to improve and maintain IPC competencies. Read about the Danish IPC activites at CEI SSI subsite[5].

EU-JAMRAI 2 receives funding from the European Union's EU4Health programme under grant agreement n°101127787.

EU-JAMRAI Joint Action Antimicrobial Resistance and Healthcare-Associated Infections

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The Danish IPC guidelines are in place

All healthcare professionals are expected to be familiar with and act in compliance with the national guidelines for IPC (published by the National Centre for Infection Control at SSI); in Danish "NIR Generelle forholdsregler for sundhedssektoren"[6]. The supplemental national guideline includes specific guidance on VRE, ESBL and other multidrug-resistant microorganisms (MDRO) and should be followed when being in contact with a patient, for which either clinical infection or carriage of MDRO is suspected or known; "NIR Supplerende forholdsregler ved infektioner og bærertilstand i sundhedssektoren"[7].

The Guidance on Preventing the Spread of MRSA by the Danish Health Authority was issued in 2006, the year MRSA became notifiable. The applicable 3rd edition of the guideline is from 2016; Guidance on Preventing the Spread of MRSA - The Danish Health Authority (sst.dk)[8]. The guideline represents the national recommendations and strategic framework for preventing the spread of MRSA at hospitals and in other healthcare settings and nursing homes. A 16-year MRSA surveillance report[9] has shown that the national MRSA strategy has been successful in controlling the spread of MRSA at hospitals as the primary goal of the MRSA guideline and to stabilize the spread of livestock-associated MRSA.

The first national guideline on preventing the spread of CPO by the Danish Health Authority was issued in 2018 (only in Danish); "Vejledning om forebyggelse af spredning af CPO"[10]. The guideline provides a national strategic framework for detection and management of CPO in hospitals. The main purpose of the guideline is to maintain a low prevalence of disease caused by CPO associated with certain high-risk situations. Despite this national guideline CPO is increasing in Denmark (see chapter 8.3.2, carbapenemase-producing organisms, CPO) indicating that more needs to be done in order to combat the outbreaks at hospitals and at long-term care facilities. There are a lot of challenges in controlling these outbreaks as they are long-lasting.

The purpose of both guidelines is to minimize the spread of these often highly resistant bacteria to the ill and weak patients at hospitals and in long-term care facilities, simultaneously keeping the occurrence of these bacteria on a continued low level. The guidelines contain recommendations for active screening of patients on admission to hospital, based on assessment of certain risk situations, e.g. admission to a hospital abroad during the last six months. Both guidelines are free of charge and easy to download from the Danish Health Authority website www.sst.dk.

In hospitals, nursing homes and home care, it is necessary to supplement the general precautions with transmission based (isolation) precautions in the case of an outbreak: "Infektionshygiejniske retningslinjer for MRSA"[11] and "Infektionshygiejniske retningslinjer for CPO"[12].

All IPC guidelines emphasize the importance of all healthcare staff, irrespective of profession, to contribute to the management and prevention of infections and to prescribe antibiotics with care.

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[1] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023H0622(01)
[2] https://eu-jamrai.eu/
[3] https://health.ec.europa.eu/funding/eu4health-programme-2021-2027-vision-healthier-european-union_en
[4] https://ec.europa.eu/info/funding-tenders/opportunities/portal/screen/opportunities/projects-details/43332642/101127787/EU4H
[5] https://hygiejne.ssi.dk/formidling/eu-jamrai-2
[6] https://hygiejne.ssi.dk/NIRgenerelle
[7] https://hygiejne.ssi.dk/NIRsupplerende
[8] https://www.sst.dk/en/english/publications/2022/Guidance-on-Preventing-the-Spread-of-MRSA
[9] https://hygiejne.ssi.dk/overvaagning/mrsa
[10] https://www.sst.dk/da/udgivelser/2018/vejledning-og-bekendtgoerelse-om-forebyggelse-mod-spredning-af-cpo
[11] https://hygiejne.ssi.dk/retningslinjer/infektionshygiejniske-retningslinjer-for-mrsa
[12] https://hygiejne.ssi.dk/retningslinjer/infektionshygiejniske-retningslinjer-for-cpo

Textbox 5.4

Consumption of antimicrobials in the Faroe Islands

Background

The Faroe Islands (FI) consist of 18 islands inhabited by approximately 54,000 inhabitants, approximately 22,000 of whom live in the capital Tórshavn. Sjúkrahúsverkid consists of the main hospital (Landssjúkrahúsid, LS, with 130 beds), located in Tórshavn, and two smaller hospitals in Klaksvík (22 beds) and Suduroy (26 beds). The Faroese healthcare system is comparable to the Danish healthcare system with general practitioners responsible for primary care and hospitals providing secondary care. LS has a local as well as a centralised function. In the case of specific diseases, demanding highly specialised care, patients are referred to hospitals in Denmark or other hospitals abroad.

Data and data sources: Data on antimicrobial consumption (purchase data) for FI and for the three hospitals were supplied by the Chief Pharmaceutical Office. Data on somatic bed-days were obtained from LS.

Antimicrobial consumption at the three hospitals

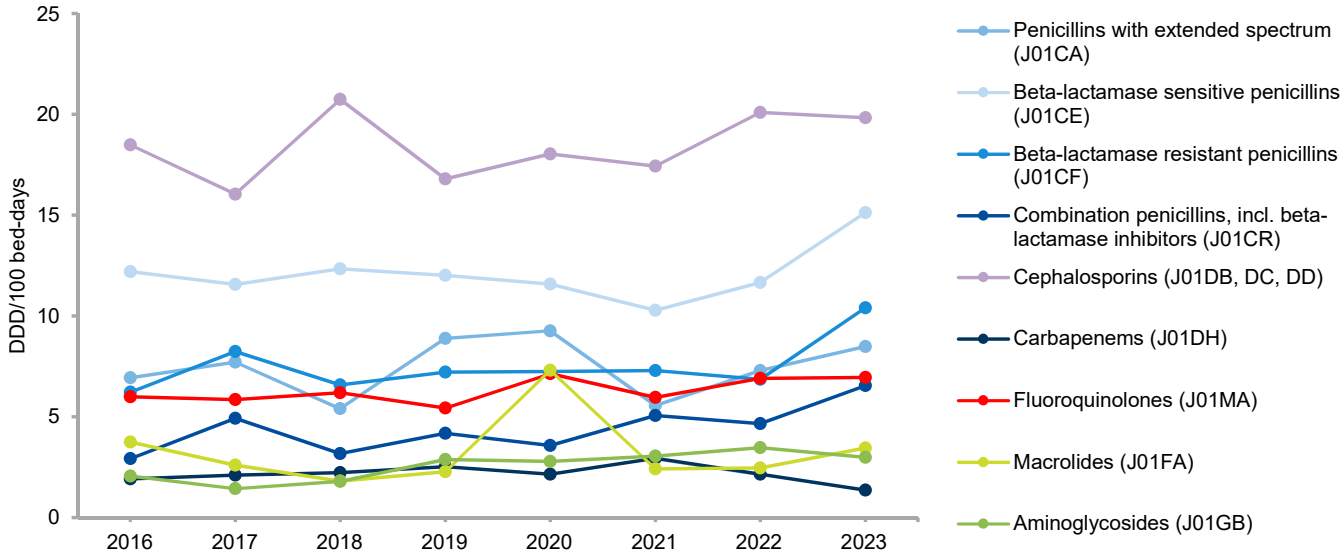
The total antimicrobial consumption was 104.13 DDD/100 bed-days (DBD), a 13% increase compared to 2022, a 26% increase compared to 2021 (82.66 DBD), and a 33% increase compared to 2016 (78.58 DBD). Special attention to three broad-spectrum antimicrobials, cephalosporins, carbapenems and fluoroquinolones is still required: In 2023, the consumption of cephalosporins was 19.83 DBD (19% of the total hospital antimicrobial consumption), slightly lower than in 2022 (20.08 DBD), but increased by 14% and 10% compared to 2021 (17.43 DBD) and 2020 (18.02 DBD), respectively. Fluoroquinolone was at the same level (6.95 DBD) as in 2022 (6.90 DBD), but has shown an increasing trend over the years with a 16% increase compared to 2016 (5.99 DBD). Problems with supply due to product shortage might be part of the explanation.

Use of carbapenems (1.36 DBD) decreased by 37% and 54% compared to 2022 (2.14 DBD) and 2021 (2.94 DBD) and is only at 71% of the level in 2016, which is a positive development.

Likewise, it is encouraging to observe a 30% increase in the consumption of beta-lactamase sensitive penicillins (15.12 DBD in 2023 vs. 11.65 DBD in 2022). A similar development is seen in the consumption of beta-lactamase resistant penicillins (10.40 DBD in 2023 vs. 6.85 DBD in 2022) (Figure 1).

Figure 1 Consumption of leading groups of antimicrobial agents at somatic departments in the three Faroese hospitals, DDD per 100 bed-days, 2016-2023

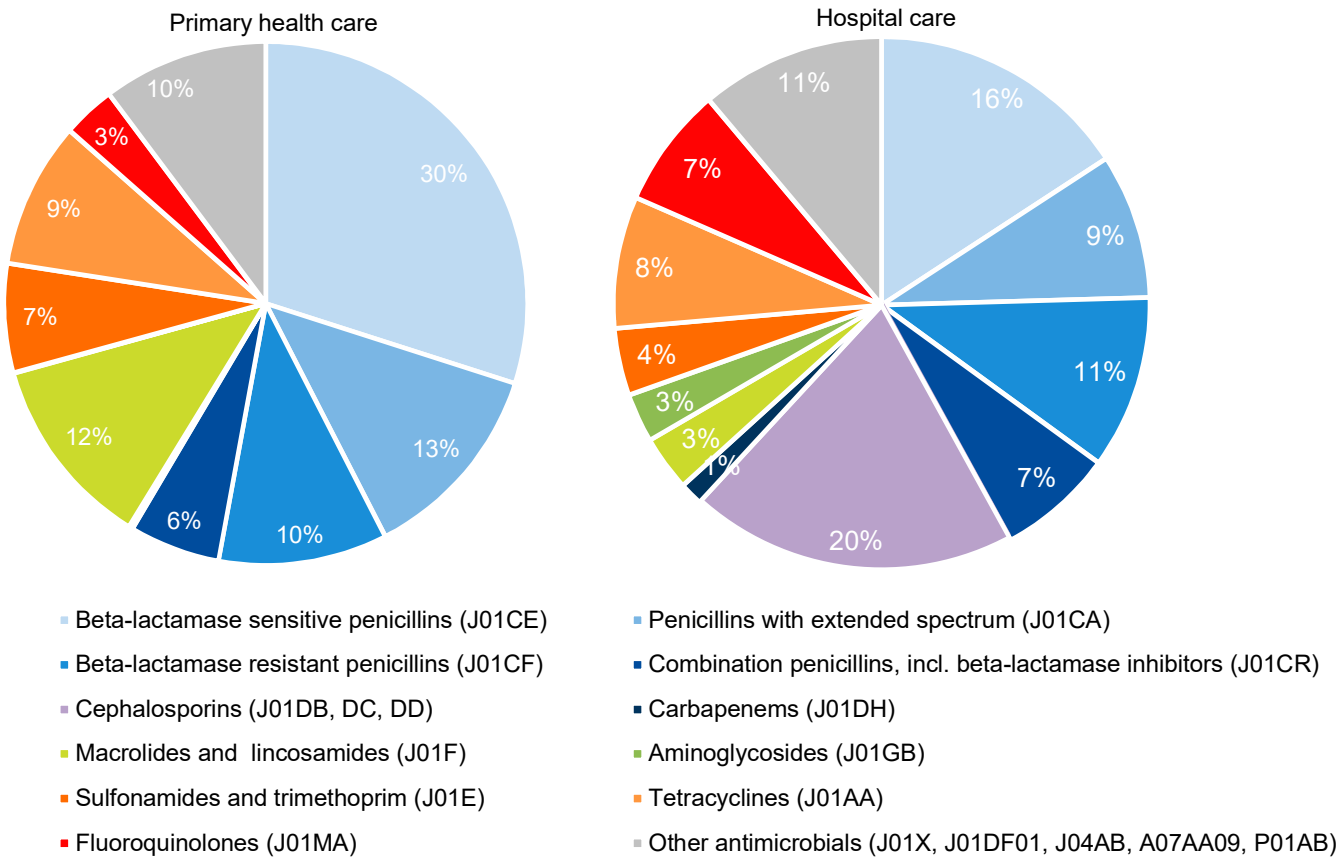
DANMAP 2023



Antimicrobial consumption in primary healthcare

In 2023, the total antimicrobial consumption in primary healthcare was 15.65 DDD/1,000 inhabitants/day (DID) – comparable to 2022 (15.26 DID), but representing a 19% increase since 2016 (13.11 DID). The distribution of antimicrobial agents used is shown in Figure 2 and compared to distribution of similar antimicrobials in hospital consumption.

Figure 2 Percentage distribution of antimicrobial agents in primary healthcare and hospital care, DDD, 2023 DANMAP 2023



Consumption of macrolides and lincosamides increased by 27% compared to 2022 (1.86 DID in 2023 vs. 1.45 DID in 2022). An encouraging observation was a 14% decrease in the use of fluoroquinolones (0.51 DID in 2023 vs. 0.59 DID in 2022).

Total antimicrobial consumption in hospitals was 2.27 DID, accounting for 13% of the total consumption at FI, whereas the consumption in primary healthcare accounted for 87%.

Resistant microorganisms

In 2023, eight patients were identified with MRSA (six were clinical specimens, two were obtained by screening). The corresponding patient numbers for VRE were two and five, respectively, and for CPO one and two respectively.

Conclusion

Focus on broad-spectrum antimicrobials as well as infection prevention and control remains crucial, as there is still a high-level consumption of antimicrobials such as the cephalosporins.

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Textbox 6.1

Trends in phenotypic- and genotypic fluoroquinolone resistance in *Campylobacter jejuni* from broilers and broiler meat in Denmark

Background

Despite annual fluctuations, the occurrence of fluoroquinolone (ciprofloxacin) resistance in *Campylobacter jejuni* recovered from Danish broilers at slaughter has increased over a decade [1]. A similar trend has been observed since 2014 in several other EU member states [2]. However, such increase is not driven by direct selection pressure, since fluoroquinolones are not used in broiler production in Denmark.

The phenotypic resistance profile and the genomic background of fluoroquinolone resistance in *Campylobacter jejuni* from broilers and broiler meat in Denmark were investigated retrospectively to identify possible shifts in resistance patterns, and to explore possible explanations for the observed trend.

Materials and methods

Two datasets were gathered for the study - one consisting of minimal inhibitory concentrations (MIC) for ciprofloxacin from 2014 to 2022 for *Campylobacter jejuni* isolates from broilers; the other containing whole-genome sequencing (WGS) results from *C. jejuni* isolates collected from broilers and broiler meat in Denmark, between 2019 and 2024. All data were provided by the Danish Veterinary and Food Administration.

The MIC dataset consisted of 1,333 *C. jejuni* isolates, comprising 1028 isolates from faecal samples from Danish broilers, and 535 isolates from broiler meat from Denmark (n=305) and other four EU countries (n=230). The WGS dataset consisted of 1,051 isolates, 24 originating from Danish broilers and 1,027 from retail broiler meat, including 808 from Danish meat and 219 from imported broiler meat from seven EU countries. Isolates from imported broiler meat were aggregated under the category "EU" for further analyses.

The multilocus sequence type (MLST) and the clonal complex (CC) were determined for 955 of the sequenced isolates. Furthermore, the total 1,051 sequences were mapped against the PointFinder database for identification of point mutations and a prediction of resistance phenotypes using the ResFinder platform version 4.5.0 [3].

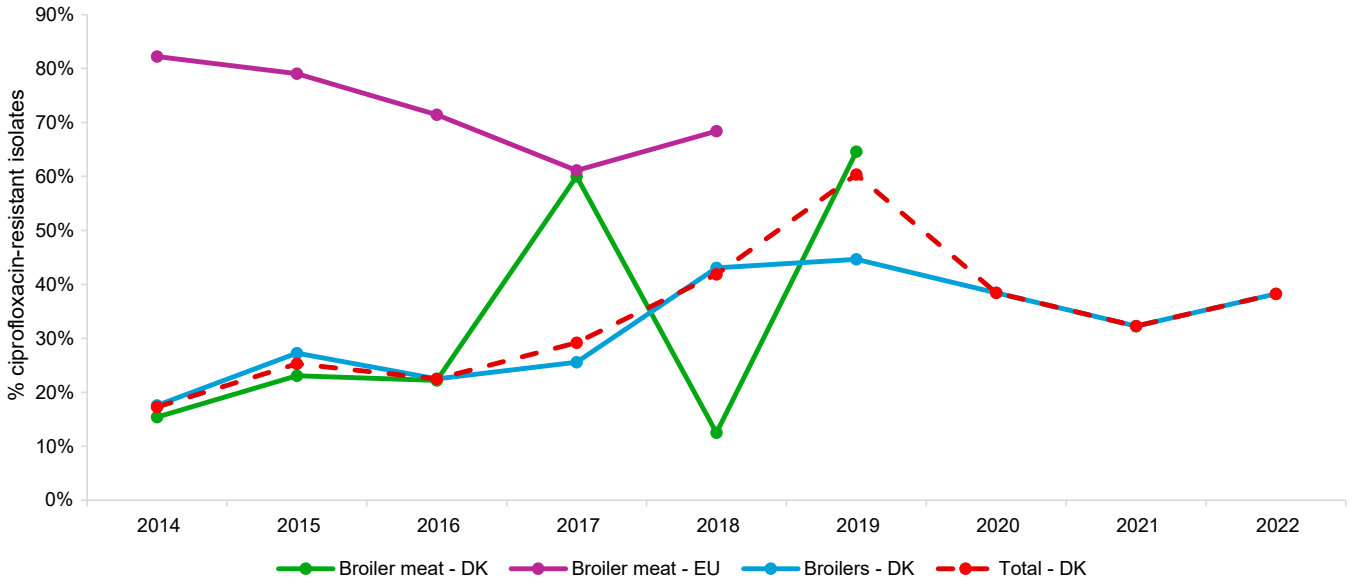
The statistical significance of the increasing trend in phenotypic ciprofloxacin resistance, and of the difference between MLST frequencies among isolates predicted as sensitive and resistant was assessed. Further analyses were performed and are available for consultation [4].

Results and discussion

Phenotypic resistance

The percentage of ciprofloxacin-resistant isolates recovered from the three different sample categories is shown in Figure 1. Between 2014 and 2016, the occurrence of ciprofloxacin resistance among isolates from imported meat was markedly higher than that observed in isolates from domestic meat. Within that period, while the first followed a decreasing trend, the latter showed an increase over time, and both reached the same value by 2017. Phenotypic ciprofloxacin resistance in *C. jejuni* from Danish broilers showed a significant increasing trend between 2014 and 2022. The same was true when considering resistance occurrence among all isolates of Danish origin (Figure 1).

Figure 1 Percent occurrence of ciprofloxacin resistance among *C. jejuni* isolates from broilers and broiler meat sampled in Denmark, 2014-2022
DANMAP 2023



The number of isolates tested from each source varied between years. The total number of isolates of Danish origin (broilers + broiler meat) tested each year were as follows: 2014 (191), 2015 (83), 2016 (178), 2017 (48), 2018 (203), 2019 (265), 2020 (164), 2021 (31), 2022 (170). In 2017 and 2018, there were less than ten isolates tested from domestic broiler meat. MIC results from *C. jejuni* isolates from imported- and Danish broiler meat were only available until 2018 and 2019, respectively

Genotypic resistance

In the period between 2019 and 2023, a total of 364 out of 1,044 (35%) *C. jejuni* isolates were predicted as quinolone-resistant, and no significant increasing or decreasing trend in the occurrence of resistance was detected, with the percentage of isolates predicted as resistant being stable at around 30-40% in the four years considered.

There were 49 different mutations of the *gyr*A gene detected among the sequenced isolates. However, only three mutations were present among isolates predicted as quinolone-resistant – predominantly mutation T86I, followed by T86A and P104S. Isolates with the mutation P104S are usually double mutants, also having T86I.

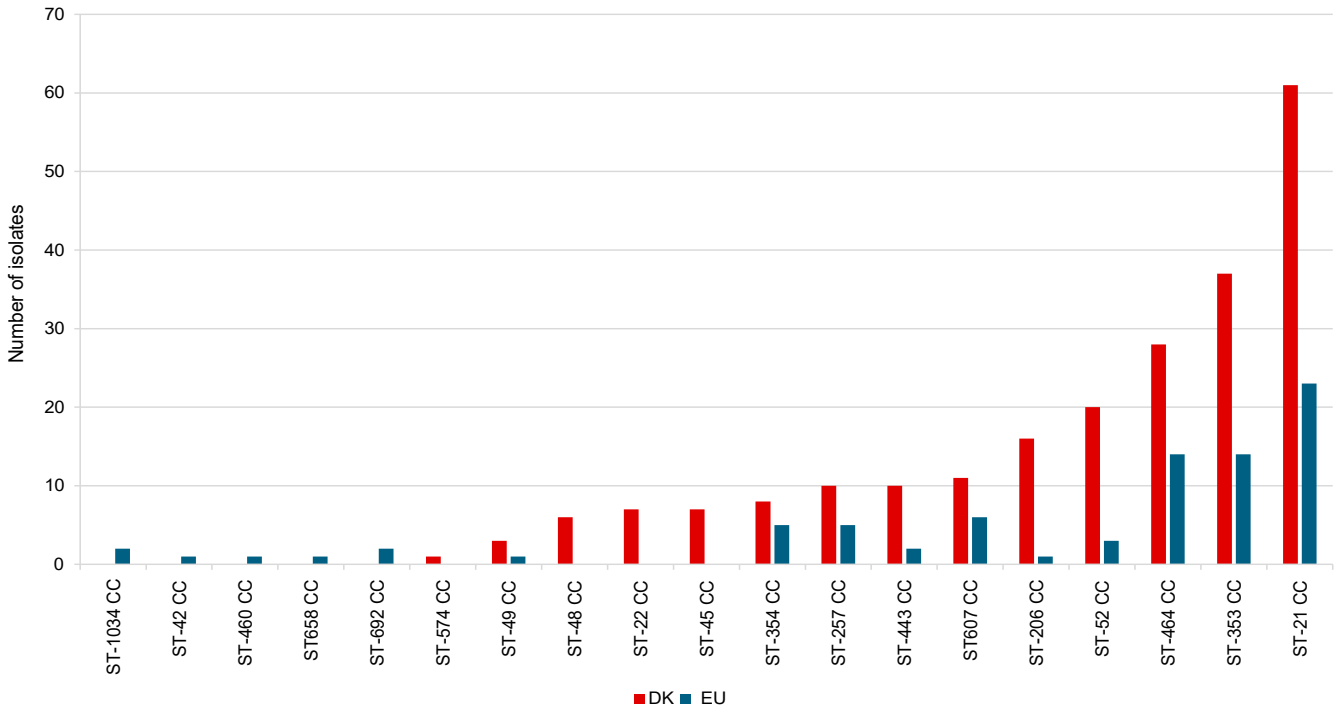
Phylogenetic typing

Among the 955 isolates with an identified sequence type (ST), 329 presented mutations in the *gyr*A gene and were predicted resistant to quinolones, belonging to 51 different STs. The relative distribution of STs was statistically different between the isolates predicted as sensitive to quinolones and those with a mutation conferring quinolone resistance.

The 51 STs identified among resistant-predicted isolates were grouped into 19 clonal complexes (CC). Fourteen CCs were found among isolates from Denmark, three of which exclusively. Likewise, five CCs were only present in isolates from imported EU broiler meat, although in very low numbers (Figure 2).

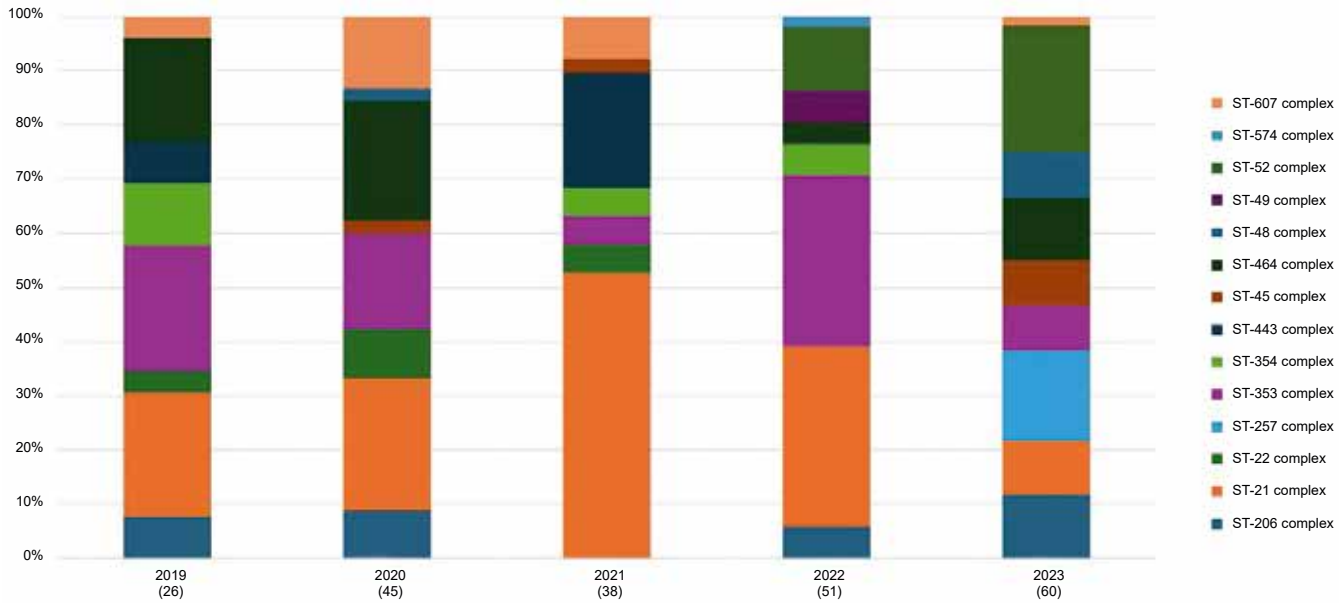
continued ... Textbox 6.1

Figure 2 Distribution of clonal complexes (CCs) among resistant-predicted *C. jejuni* isolates of Danish- and EU origin DANMAP 2023



The distribution of CCs among resistant-predicted isolates of domestic- and EU origin were mostly similar, with the exception of CCs ST-52 and ST-206, which were the fourth and fifth most common among Danish isolates, respectively, but less common among isolates from imported meat. The distribution of CCs among resistant *C. jejuni* mutants from Denmark between 2019 and 2023 is shown in Figure 3.

Figure 3 Percentage of clonal complex abundance among *C. jejuni* resistant mutants of Danish origin, 2019-2023 DANMAP 2023



Total number of resistant-predicted isolates per year is shown in parentheses

While predominant clonal complexes such as ST-21 CC and ST-353 CC appeared every year as expected, other CCs such as ST-45 CC, ST-48 CC, ST-49 CC and ST-574 CC were observed only occasionally. The relative distribution of CCs among quinolone resistant-predicted isolates from Denmark appears more balanced in 2023 compared to previous years, with an increased presence of ST-206, ST-22, ST-48 and ST-45, and a decreased presence of ST-21 CC and ST-353 CC. Interestingly, in 2023, ST-52 CC was the most abundant among Danish resistant mutants ST-52 has been associated with a recent long-lasting outbreak of human campylobacteriosis in Denmark [5], which began in 2021. A phylogenetic analysis comparing ST-52 resistant mutants from broilers and the outbreak ST-52 isolates would be of interest to investigate whether resistant *C. jejuni* has been involved in the outbreak. Also, in 2023, the ST-257 complex appeared for the first time during the period considered. In a recent retrospective study of *C. jejuni* isolates from human clinical cases in the United Kingdom, the clonal complex ST-257 appears among many CCs that showed an increase in the levels of fluoroquinolone resistance over the last two decades [6].

Conclusion

The phenotypic analysis confirmed the existence of an increasing trend of ciprofloxacin-resistant *C. jejuni* isolates in Danish broilers over the last decade, however this trend was not observed for isolates predicted as quinolone-resistant based on the presence of a *gyr*A point mutation. Further studies are needed in order to investigate the cause for this discrepancy.

The phylogenetic typing of the resistant-predicted isolates from Danish broiler meat revealed a shift in variability in 2023, including an increased presence of the clonal complexes ST-52 and ST-257, compared to previous years. Previous studies have shown that resistance patterns within certain clonal complexes can change over time.

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Textbox 7.1

Ecogenomics of Danish cattle *E. coli* between 2001 and 2019

Background

The genomic evolutionary trends of antimicrobial resistance and virulence in indicator *Escherichia coli* within Danish cattle from 2001 to 2019 were recently investigated, focusing on the prevalence, distribution and evolution of different strains. Specifically, the study focused on changes in antimicrobial resistance genes, virulence genes, and phylogeny, and what factors might have contributed to such changes in *E. coli* from Danish cattle.

Materials and methods

The study employed whole genome sequencing of 1,359 indicator *E. coli* isolates from 598 Danish cattle farms, followed by the profiling of their antimicrobial resistance and virulence genes. Furthermore, correlations of resistance and virulence profiles to either year of collection or farm of origin were also explored. Lastly the phylogenetic relatedness among *E. coli* isolates was investigated to draw their ecogenomic signature in cattle over time and space.

Results and discussion

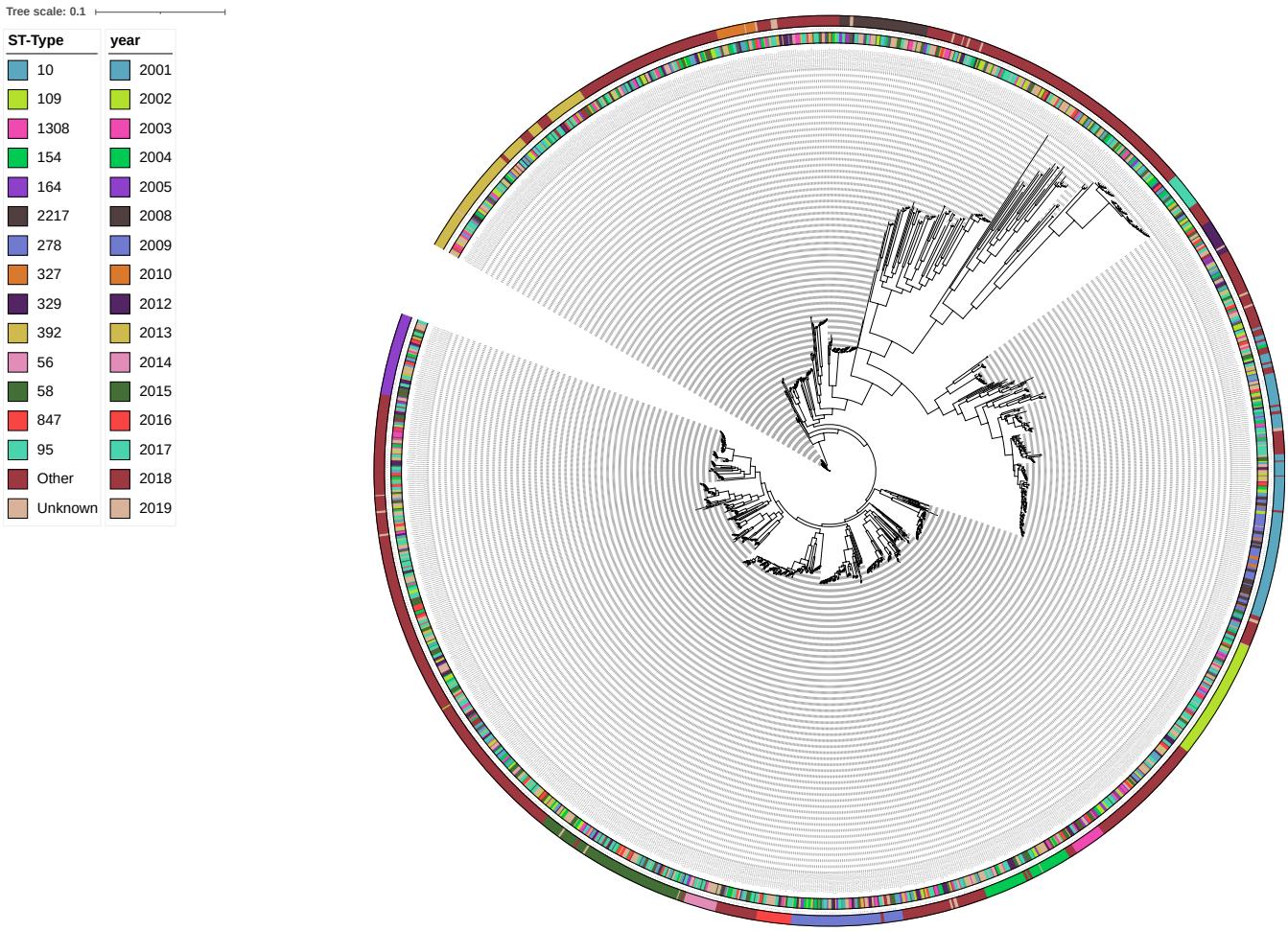
The results showed a rich genomic landscape characterised by the presence of 263 different sequence types among the analysed *E. coli* isolates. The phylogenetic analysis further highlights substantial genetic diversity, with clustering primarily based on sequence types rather than temporal and spatial factors (Figure 1).

Subsequently, we separated the *E. coli* isolates into two groups to create a more homogeneous distribution of number of isolates across farms and reduce biases associated with frequent collections from some locations. Genome-wide comparisons of the isolates indicated that a few single-copy orthologs may exhibit a spatio-temporal pattern, although the significance did not suggest a strong association. Notably, there was a significant reduction in the occurrence of certain genes primarily related to DNA transfer. This pattern was more pronounced in isolates collected after 2015, indicating that the temporal divide between the two groups likely falls within the 2014-2015 period. We are currently investigating the reasons for such separation before and after this period.

The antimicrobial resistance analysis revealed that 22% of isolates harbour resistance genes, with the highest rates observed in the aminoglycoside (15%), beta-lactam (11%), folate pathway antagonist (11%), and tetracycline (12%) classes. Correlation analyses showed diverse resistance gene dynamics across the years, with notable deviations in 2008-2010 and 2014, where there was an increase in resistance observed across multiple antimicrobial classes. Virulence analysis identified key virulence genes associated with the pathotypes enteropathogenic *E. coli* and Shiga toxin-producing *E. coli*.

Further results of this study are available for consultation [1].

Figure 1 Overview of phylogenetic relationships between isolates, serotype and collection year (color-coded) DANMAP 2023



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Textbox 8.1

Danish surveillance of azole resistant *Aspergillus fumigatus* from clinical samples – a 4-year update

Azole resistance in *Aspergillus fumigatus* (AR*Af*) has increased worldwide. The most efficacious and the only orally available drug class is the azoles [1]. A significant and growing proportion of resistance has emerged in the environment due to the widespread use of azoles in agri- and horticulture. This can lead to infections in azole naïve patients, where resistance may not be anticipated. This "environmental" resistance is characterised by a combination of a sequence duplication in the promoter region of the *cyp51A* gene coupled with one or several point mutation(s) [2,3]. The most common mechanism is TR_{34}/L98H, which causes pan-azole resistance, whereas TR_{46}/Y121F/T289A results in high voriconazole and isavuconazole MICs. Delayed appropriate treatment for invasive aspergillosis caused by these isolates has been associated with significantly increased mortality. Consequently, an international expert group has advocated for the use of alternative empiric treatment when the local resistance rate exceeds 10% [2,4].

A voluntary Danish Nationwide surveillance of azole resistance in clinical *A. fumigatus* isolates was initiated in October 2018 [5]. Clinical isolates of *A. fumigatus* were susceptibility tested using the EUCAST method and azole resistant isolates were *cyp51A* sequenced. Repeat isolates from individual patients were excluded if ≤30 days apart.

During the first four years, a total of 3,455 isolates from 2,543 patients were included. Overall, 5.8% (201/3455) of isolates were resistant to at least one azole (between 5.2% to 5.4% for the individual azoles). In 3.0% (105/3455) of isolates, resistance was caused by environmental mechanisms (Table 1). Of the 137 patients with AR*Af*, 58.4% (80/137) harboured isolates with environmental resistance mechanisms, whereas a plethora of other alterations were detected among the remaining (Figure 1). Resistant isolates without Cyp51A alterations were also identified (Table 1, Figure 1). TR_{46} variants were exclusively found in year three. Almost half of the patients with AR*Af* (45% (62/137)) also harboured susceptible isolates the same year. This underscores the importance of sequential testing to detect isolates with different susceptibility patterns over time.

Increased resistance over the four-year period was not found (Chi square time trend p>0.5) but continued surveillance over time is required as resistance may fluctuate and can increase only gradually [6,7] (Figure 2). In the Netherlands, resistance rates have increased (from 7.6% in 2013 to 14.7% in 2018), whereas resistance rates in Denmark remain below the 10% threshold. *A. fumigatus* is abundant in the environment and is acquired breathing ambient air. A Danish environmental study from 2020-2022 demonstrated ARA*f* in 4.2% of 4,538 environmental *A. fumigatus* isolates. Resistance was mainly caused by TR_{34} (79.4%) and TR_{46} (11.3%) genotypes, the last of which was only detected in 2021-2022 [8]. This is slightly higher than the environmental resistance rate in patients, suggesting a future increase in resistant infections may be expected, and it correlates with the fact that TR_{46} was only detected in patients from the third surveillance year.

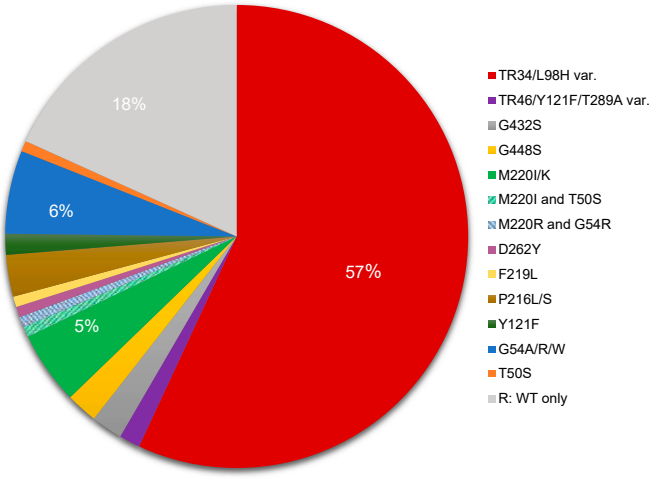
Dual use of same-class active agents in both clinical drugs and environmental pesticides also poses a risk to future drugs under development that could be used to treat azole resistant infections [9]. Regulatory efforts and continued surveillance are still needed.

Table 1 Yearly number of isolates and patients DANMAP 2023

	Quarter and year of surveillance									
	1 Q4 2018-Q3 2019		2 Q4 2019-Q3 2020		3 Q4 2020-Q3 2021		4 Q4 2021-Q3 2022		All Q4 2018-Q3 2022	
	*N**	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Patients	675		562		688		618		2,543	
Isolates	978		843		883		751		3,455	
Susceptible	922	94.3%	782	92.8%	832	94.2%	718	95.6%	3,254	94.2%
Resistant	56	5.7%	61	7.2%	51	5.8%	33	4.4%	201	5.8%
R env.	35	3.6%	21	2.5%	32	3.6%	17	2.3%	105	3.0%
R other Cyp51A	14	1.4%	21	2.5%	14	1.6%	11	1.5%	60	1.7%
R non-Cyp51A**	7	0.7%	19	2.3%	5	0.6%	5	0.7%	36	1.0%

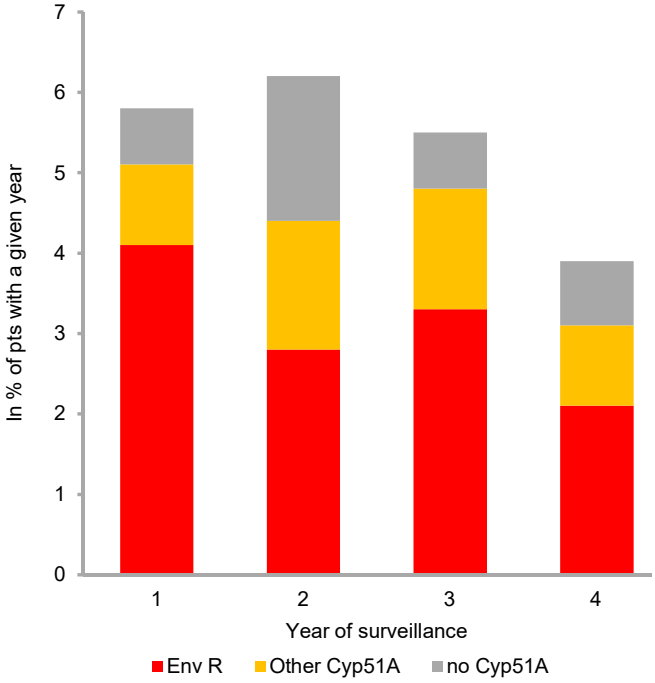
R env.: Resistance due to alterations of environmental type. *For year 1, isolates that had not been sequenced (five isolates/three patients) but were phenotypically similar to same-patient isolates with *cyp51A* mutations were counted as the given mutation. **For year 2, one resistant isolate had an N248K mutation which is not linked to azole resistance

Figure 1 Cyp51A alterations found in 137 patients with azole resistance DANMAP 2023



R: WT denotes patients with resistant isolates without demonstrated *cyp51A* mutations. Var: TR_{34}/L98H and TR_{46}/Y121F/T289A isolates may have additional alterations (not shown). Some patients harbouring isolates with *cyp51A* mutations also have susceptible or resistant isolates without mutations within the same year (not detailed). The resistant isolate with an N248K alteration is shown under R:WT

Figure 2 Resistance on a patient level over the four-year period DANMAP 2023



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Data has in part been presented as a poster P2917 at the ESCMID Global 2024 in Barcelona, Spain.

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Textbox 8.2

Increasing rates of drug resistance in *Mycobacterium tuberculosis* isolates in Denmark

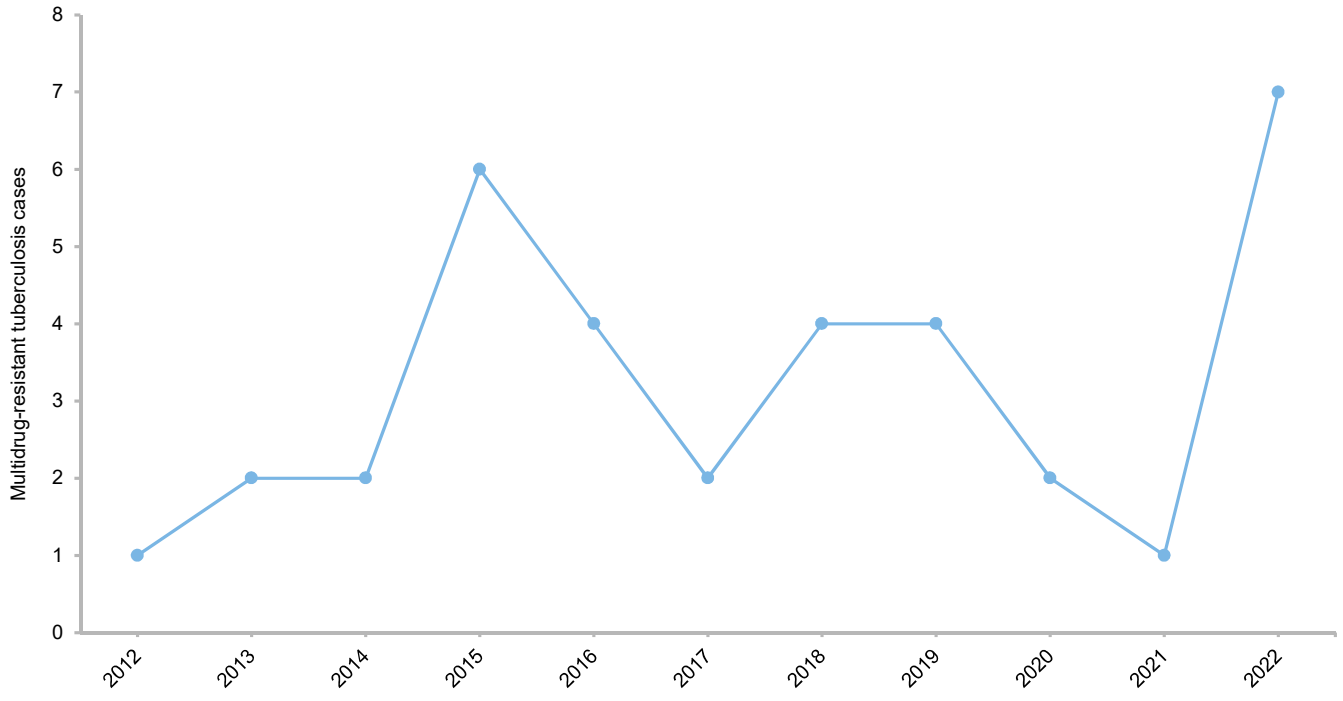
Background

In recent years, the global incidence of tuberculosis (TB) has declined. However, the emergence of drug-resistant Mycobacterium tuberculosis (Mtb) strains poses an urgent challenge to TB control in many parts of the world. It is estimated that only two out of five people with drug-resistant TB obtain the proper treatment (https://www.who.int/news-room/fact-sheets/detail/tuberculosis). Multidrug-resistant (MDR) TB is caused by Mtb resistant to at least rifampin (RIF) and isoniazid (INH), the two most important drugs for TB treatment. Extensively-drug-resistant (XDR) TB is a form of MDR-TB with additional resistance to any of the fluoroquinolones (e.g. moxifloxacin) and at least one other group A drug (bedaquiline or linezolid). Mtb drug resistance is caused by spontaneous gene mutations during bacterial multiplication, which confer resistance to the drugs at different frequencies. Exposure to a single drug or suboptimal drug concentrations provides a selective environment favouring drug-resistant bacteria and the development of MDR/XDR-TB [2]. For many years, MDR-TB was seen in 2-3 cases a year in Denmark, with a rise in 2015 with five cases in a Danish outbreak[1], even though the majority of MDR/XDR TB in Denmark is seen among immigrants.

Methods

In Denmark, all clinical samples from suspected TB patients are cultured at the International Reference Laboratory of Mycobacteriology, Statens Serum Institut (SSI). Fluorescent microscopy of acid-fast auramine-rhodamine stained samples and for every new patient, an Mtb PCR including genotypic results for the two most critical first-line drugs RIF and INH. Subsequently, the samples are cultured in liquid and solid media. If the culture is positive for Mtb, resistance detection and subtyping are performed by WGS. If any drug resistance mutations are present, phenotypic susceptibility testing is performed by the modified proportion method in liquid media in MGIT 320/960 Systems (BD).

Figure 1 MDR-TB cases microbiologically verified in Denmark from 2012-2022 DANMAP 2023



Results

Seven MDR-TB cases were detected in Denmark in 2022 among 232 notified cases, corresponding to 3% MDR-TB total (Fig. 1). Additionally six cases (4%) were isoniazid mono-resistant.

Discussion

The 2022 drug-resistance figures for Mtb are the highest ever registered in Denmark with seven laboratory-confirmed MDR-TB cases plus, to the knowledge of the laboratory, at least five instances of immigrants entering Denmark with MDR-TB. Although the total figures are small, we must be prepared for an increasing number of future MDR/XDR-TB cases in Denmark. It is vital to remind clinicians to be aware of drug-resistant tuberculosis and to send all specimens for culturing, among others, to secure sensitive, fast and correct susceptibility testing performed through a combination of phenotypic- and genotypic test methods. The sensitivity of genotypic tests to detect RIF and INH resistance mutations is high. Therefore, susceptibility towards these drugs can be trusted based on the absence of resistance-conferring mutations[2]. Compared with detecting resistance-conferring mutations to the first-line drugs, the knowledge of which mutations are causing resistance to second-line drugs is lower. Hence, the more time-consuming phenotypic susceptibility testing is still essential for identifying the correct regime.

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Textbox 8.3

First results from antimicrobial resistance monitoring in *Shigella* spp. in Denmark

Shigella is an enteroinvasive bacterium causing shigellosis, a gastrointestinal infection in humans characterized by diarrhea, abdominal pain and general malaise.

The genus *Shigella* includes four species, *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. The predominating *Shigella* species in Danish patients are *S. sonnei* and *S. flexneri*. Infections occur through fecally contaminated food or drinking water and are therefore particularly associated with poor sanitation and poor food hygiene, but may also occur via direct contact, e.g. among children in day care or in adults through sexual contact. Shigellosis is a self-limiting infection that typically lasts five to seven days. In more severe cases or in case of prolonged diarrhea the recommended antibiotic is azithromycin. Infections caused by *Shigella* or Enteroinvasive *Escherichia coli* (EIEC) are mandatory reportable in Denmark.

In 2023, 717 cases of EIEC were registered in Denmark. The primary PCR-based diagnostics do not distinguish between *Shigella* spp. and EIEC, but the species is determined for the subset of cases where an isolate is referred to the reference laboratory at SSI.

In 2023, the reference laboratory at SSI received 92 *S. sonnei* and 30 *S. flexneri* isolates from Danish patients. Travelling abroad before onset of disease was recorded for 58% (53/92) of the *S. sonnei* and 37% (11/30) of the *S. flexneri* cases. The remaining cases were not travel related or lacked information about travel status. The isolates were tested for antimicrobial susceptibility by determination of the minimal inhibitory concentration (MIC) and interpreted using epidemiological cut off values. Further 67 *S. sonnei* and 14 *S. flexneri* isolates were subjected to whole-genome sequencing (WGS).

The results of the phenotypic susceptibility testing are presented in Table 1.

Table 1 Resistance (%) in *S. sonnei* and *S. flexneri* isolates from Danish patients in 2023 DANMAP 2023

Antimicrobial agent	*S. sonnei*	*S. flexneri*
Amikacin	0	0
Ampicillin	67	90
Azithromycin	30	23
Cefotaxime	62	10
Ceftazidime	20	3
Chloramphenicol	0	63
Ciprofloxacin	84	50
Colistin	0	0
Gentamicin	0	3
Meropenem	0	0
Nalidixic acid	47	33
Sulfamethoxazole	83	50
Tetracycline	77	87
Tigecycline	0	0
Trimethoprim	100	77
Fully sensitive (%)	0	3
Number of isolates	92	30

Most *Shigella* isolates were resistant to a number of different antimicrobials. Azithromycin resistance levels of 30% and 23%, respectively, were observed in *S. sonnei* and *S. flexneri*, and the WGS data showed that all sequenced resistant strains harbored the *mph(A)* gene.

The levels of quinolone resistance were notably high, 84% and 50% respectively for *S. sonnei* and *S. flexneri*, and it is noteworthy that the levels of fluoroquinolone resistance were higher than the levels of nalidixic acid resistance. The sequence analysis showed that *Shigella* frequently harbors *qnr* gene variants on plasmids.

Resistance towards 3rd generation cephalosporins are commonly observed in *Shigella*. Cefotaxime resistance was observed in 62% of the *S. sonnei* isolates and in 10% of the *S. flexneri* isolates. Sixteen of 61 patients who were infected with a cefotaxime resistant *Shigella* had no history of travel. Cefotaxime resistance was in most cases mediated by the *blaCTX15* gene, but *blaCTX-M-27* and *blaCTX-M-231* were also identified.

Carbapenemase (meropenem) resistance was not observed in any of the tested isolates and the levels of aminoglycoside, amikacin and gentamicin resistance was low. Colistin resistance was not observed.

Chloramphenicol resistance was frequent in *S. flexneri* of which 62% of the isolates were resistant, whereas chloramphenicol resistance was rare in *S. sonnei*.

The levels of resistance observed in the Danish *Shigella* isolates were generally in concordance with the levels that are reported internationally.

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